MITOSIS OF THE PRIMARY NUCLEUS IN SYNCHYTRIUM DECIPIENS.

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(WITH PLATES XVI AND XVII)

THE fungus *Synchytrium decipiens* Farlow invades single cells of the hog peanut (*Falcata comosa* (L.) Kuntze) and there causes proliferation of the tissue until the host cell is imbedded in a gall of considerable size. The parasite is at first seen resting in the cytoplasm of the host cell and occupying it conjointly with the host nucleus of that cell. The parasite, growing more rapidly than the host cell in the early stage of its enlargement, soon comes to occupy the whole cell space, while the host nucleus slowly disappears. During further gall growth the parasitized cell becomes many times larger than when attacked. The cytoplasm of the parasite increases *pari passu*, continuing to fill completely the host cell. Increase in cytoplasm is accompanied by a corresponding growth of the Synchytrium nucleus. It thus happens that while the nucleus of the invading Synchytrium was at first very small, it later reaches proportions vastly larger than the nucleus of the host plant, larger even than the notoriously large nuclei found elsewhere in the plant kingdom. The maximum diameter of the embryo sac nuclei of flowering plants ranges in the neighborhood of 20–30 μ and that of the vegetative nuclei from 5 to 9 μ. The nucleus of this unicellular fungus parasite often attains a diameter of 35 μ, with a nucleolus 11 μ or more in diameter.

The vegetative period of the parasite is characterized by increase in size of both the fungus body and its nucleus. This period may be said to end and the reproductive period to begin with those processes which lead to separation of this mass of cytoplasm into numerous portions which are by further division to become swarm spores. The first step toward the separation of the cytoplasmic body is the division of this primary nucleus.

This process is of peculiar interest because of the large size of the nucleus, quite exceptional among the fungi, its peculiar rapid growth and subsequent division, and the problematic taxonomic position of the Chytridiales, which we may reasonably hope will be cleared up by cytological research. The very few investigations already published on species of this genus in no way detract from the interest of the problem. Two investigators, Dangeard ('90) and Rosén ('93), have reported results from *Synchytrium Taraxaci* DeB. & Wor., which, though not agreeing in detail, reveal conditions entirely unique. They are of such a nature as to demand further investigation and if possible reconciliation with current theories of the nucleus, which suffer violence if the conditions imperfectly reported by these authors really exist. Dangeard employed modern technique, although absolute alcohol and haematoxylin, his favorite fixative and stain, do not seem to be adapted to critical research in this group.

Dangeard describes the nuclear membrane of *Synchytrium Taraxaci* as granular, the nucleolus also being granular and quite spherical. He says that the nucleus divides by successive bipartition, a method of direct division. The membrane inflexes and the daughter nuclei become separated by constriction of the parent nucleus. He figures such division, showing two daughter nuclei together with their clumped chromatin masses grouped on adjacent sides, the parent chromatin group being evidently constricted, as is the remainder of the nucleus. Aside from this curious method of direct division, Dangeard states that the nucleus sometimes divides indirectly, and his *fig. 23* shows what he considers to be a type of mitosis. From both his text and figures it appears that this mitosis occurs in divisions later than the primary, and that both mitosis and direct division may occur side by side in the same cytoplasm, thus presenting a very unique condition, since it is usual in multinucleate masses of cytoplasm for the nuclei to divide not only by the same mode but also almost simultaneously. Dangeard does not claim to have followed the mitosis in Synchytrium through its phases, but he mentions it rather incidentally as a mode seen but not followed in edtail.

Rosén ('93) touched lightly upon *Synchytrium Taraxaci*, and his results agree with those of Dangeard to the extent that he describes a direct division in the primary nucleus. This division however is so different in type from that described by Dangeard that it certainly could not have been described or figured from the same structures. In the primary nucleus Rosén finds that the chromatin loops into a spirem, the nucleolus divides, the halves migrating to the forming daughter nucleus. The nucleus then constricts in the middle, thus completing a division of the nucleus in its spirem condition without the aid of the usual achromatic structures. He asserts that as successive divisions follow they assume more and more the character of mitosis, eventually presenting a typically mitotic division.

While discrepancies exist between these two authors regarding the details of the amitosis, they agree that mitosis is the exception and amitosis the rule; also that the primary division, *i. e.*, the first division of the one primitive nucleus of each sorus, is a direct division.

The present investigations have been exclusively concerned with *S. decipiens* Farl., and our results are directly comparable therefore with those of Dangeard and Rosén only in so far as different species of one genus may be expected to agree in cytological detail. However, the work on Fucus (Oltmanns, '89) and Albugo (Stevens, '99, '01) shows more specific cytological variation than *a priori* judgment would admit.

The caption of the present article, indicating that the primary division is mitotic, emphasizes one of the chief features of divergence between these results and those of Dangeard and Rosén. We may here indicate also that in affirming that the primary division may be mitotic we in no way set aside the possibility of its being sometimes, even frequently, amitotic. There are many peculiar structures to be found in the sori of Synchytrium, which it seems impossible to reconcile with universal mitotic division. A consideration of these leads to conclusions at variance again with those of Dangeard and Rosén regarding the details of the amitotic division. Discussion of these structures is reserved for a separate paper, the present one being lim-

ited to a description of a series of stages, all clearly pertaining to true mitosis in the primary nucleus.

De Bary and Woronin observed and have described in beautiful detail the entrance of Synchytrium into the host cell. From this point it is easy to follow the cytological changes. The cytoplasm of the invading parasite stains deeply, and is clearly recognized imbedded in the lighter cytoplasm of the host cell. For some time both the nucleus and the cytoplasm of the host cell persist, but eventually they disappear, the parasite in the meantime increasing rapidly in size. A moderately early stage of development is represented in *fig.* 1, which shows the granular, darkly staining mass of cytoplasm, spherical in form, and with a relatively large nucleus and nucleolus. Adjacent host cells exhibit nuclei and chloroplasts, thus admitting of comparison as to their relative size. At this and other early periods of development the nucleolus is large, usually solitary, homogenous in appearance, and surrounded by a thick darkly staining wall which is probably largely composed of a layer of linin laden with chromatin. Encasing the nucleolus is a mass of chromatin usually forming a continuous covering, and in places collected into irregular heaps and lumps. Occasional strands of chromatin connect the nucleolus, which is usually centrally placed, with the nuclear membrane. Chromatin is also found distributed in apparently disconnected globules of varying size, studded thickly over the inner surface of the nuclear membrane.

The parasite rapidly enlarges to occupy completely the host cell, which soon becomes enormous in size as gall-formation proceeds. A single host cell which normally averages less than 15 μ in diameter after occupation by the parasite usually attains a diameter of 100 μ or more. As the cell grows the nucleus grows, reaching at its maximum a diameter of 35 μ. During this enlargement the character or structure of the nucleus changes somewhat. The nuclear membrane becomes thicker and more conspicuous. The globules of chromatin studding its inner surface increase in number and size, and more connections are established between the chromatin surrounding the nucleolus and that of the periphery.

The nucleolus follows the growth of the nucleus, enlarging to a diameter of about 14 μ. Nucleolar inclusions in the form of homogenous globules of varying size imbedded in a granular matrix are more abundant than in earlier stages. A single cell of Synchytrium divested of its host envelope is exhibited in *fig. 2*. The nucleus has not yet reached full size, but is typical of this stage of development. It shows well the thick nuclear membrane, the peripheral chromatin, the connecting strands, and the nucleolar inclusions. *Fig. 3* shows a single more mature nucleus drawn to a larger scale. Here the chromatin is arranged in lumps, massed largely on one side of the nucleolus, while the inside of the wall is thickly beset with chromatin-bearing globules. The nucleolus is still more granular and the inclosed globules more numerous than in earlier stages. The whole dark central portion of the nucleolus must be interpreted as a vacuole, which, together with the increasing number and size of imbedded globules (dissolution products), indicates the disintegration of the nucleolus, a change further emphasized in *fig. 4*, where the nucleolus is almost entirely converted into the characteristic vacuoles. This change in the nucleus is the first visible sign of approaching mitosis. In the stages represented in *figs. 1, 2, 3, 4*, a large and remarkably clear area may be seen between the nucleolus and nuclear membrane, evidently a large vacuole created by the characteristic aggregation of the chromatin.

Up to this time the nuclear membrane has been sharp and distinct, even thick, the nucleolus large and conspicuous, the chromatin in irregular masses partaking in no way of the appearance of threads, and a large intranuclear vacuole has been constantly present.

All of this now changes. The membrane becomes gelatinous, the chromatin assumes a spirem form, and the nucleolus disappears. The nuclear membrane, previously thick and definite, first loses sharpness on its outer surface and is no longer to be seen as a definite wall. Dissolution begins from the outside. The nuclear region is long maintained, clearly mapped out, being now bounded by a layer of gelatinous consistency which stains more darkly with the orange G than does the surrounding

cytoplasm. The replacement of the nuclear wall by this gelatinous substance is a process which can be followed closely from its inception (*fig. 5*) to the later stages of mitosis (*fig. 14*). The inner boundary of the nuclear wall remains definite until mitosis is well advanced (*fig. 10*). The first indication of the dissolution of the nuclear wall is evidenced by the darker staining of the cytoplasm immediately in contact with the wall (*fig. 4*). This darkly stained region rapidly grows to a layer of gelatinous consistency in which the cytoplasm is of finer mesh and tends to be more granular. Changes very similar to those noted accompany both dissolution and building up of walls in Albugo (Stevens, '99). As the character of the nuclear membrane changes a marked shrinkage in the size of the nucleus occurs, possibly directly induced by the altered osmotic relations. A decrease from 40 to 20 μ is not unusual in this first step toward mitosis. The nucleolus, dissolution of which had proceeded far in *fig. 3*, now completely disappears. The line bounding it, which seemed a husk of chromatin rather than a definite membrane, vanishes, and the nucleolar substance, which was much wasted by the one large and many small vacuoles, is no longer to be seen. Occasional small globules, staining like vacuoles of the nucleolus (*fig. 3*), may be found in the spirem and constitute the only remaining trace of the nucleolus (*fig. 7*).

The chromatin undergoes a change as striking as that of the membrane and nucleolus. Formerly coarse and lumpy (*fig. 3*), its globular masses become much more numerous and relatively smaller (*fig. 5*). They then appear to elongate, the numerous globules being replaced by rods crossed and tangled in inextricable confusion. *Fig. 6* represents a condition where the globules have partially changed to the elongate form, while *fig. 7* shows the completion of this phase, resulting in what must be regarded as the typical spirem of this primary division in *Synchytrium decipiens*. It is characterized by fine even threads of chromatin, uniformly distributed throughout the nucleus, yet tangled and intertwined in a most complicated way. The dots in the figure represent end views of the chromatin threads, and the lines the same from a side view. The slightest change in focus brings

many others to view. It is particularly noticeable that no curving or looping is seen, the threads ever remaining straight and intersecting in acute angles.

Judging merely from the size of the nuclei, the critic may assert that *fig.* 7 represents a stage intermediate between those of *figs.* 1 and 3, a criticism that is fully met, however, by further study of the figures, since neither the condition of the nuclear membrane nor of the nucleolus admits of the intercalation of any such condition as that shown in *fig.* 7. Moreover, the sorus is at its maximum when presenting the structure shown in *fig.* 7, which would not be the case if this were intermediate between *figs.* 1 and 3. Even if no more advanced stages were discovered, we see no escape from the conclusion that *fig.* 7 represents the spirem and is a later development from such structures as are shown in *figs.* 1, 2, 3, etc. The fact that *fig.* 6 is slightly smaller than *fig.* 7 is explicable by the assumption that it is derived from a smaller resting nucleus. *Fig.* 6 clearly represents a stage early in the dissolution of the nuclear membrane, likewise early in spirem formation. It must lie between *figs.* 5 and 7, and that without contradicting the general fact of a nuclear shrinkage throughout the mitosis. *Figs.* 8–10 represent a progressive series of stages clearly more advanced than *figs.* 4–6. In these the boundary region is practically unchanged in character, though continually contracting with the diminution of the nucleus. The spirem threads as shown in *fig.* 8 become slightly thickened, apparently by longitudinal fusion of separate rods. There is a tendency of the chromatin to accumulate in masses, though never partaking of that peculiar characteristic lumpy appearance shown in *figs.* 3, 4. Vestiges of the nucleolus remain as in *fig.* 7 throughout further stages of mitosis. *Fig.* 9 shows, in a much more pronounced way, how the threads coalesce as they meet in the center. Meanwhile the size of the nucleus decreases. In *fig.* 10 there is a distinct indication of an arrangement in spindle form, though many strands remain as yet apparently in no way connected with the developing spindle. From this series and numerous other similar stages seen by the writers, there remains no doubt that from the spirem the nucleus passes to a definite

spindle formation. This spindle is intranuclear, thus agreeing with the nuclei of fungi generally. However, it is usual in the intranuclear formation of the spindle for the poles to originate in contact with the nuclear membrane, whereas in this case they are far from being so placed. As to the exact mode of spindle formation we can say nothing further than that the threads of the spirem group form themselves into a spindle. No centrosomes or polar radiations were distinguished in any stages of the mitosis.

During progress from the condition shown in *fig. 3* to that of *fig. 10*, a striking and remarkable change has occurred, in that the whole chromatin content has decreased largely. *Figs. 12* and *14* show a still greater reduction. All of the chromatin-bearing parts diminish, until, as in *fig. 11*, only a well marked spindle remains, bearing a few short chromosomes. In all early stages there seems to be a vast quantity of chromatin distributed at first throughout these globules, and later (*fig. 7*) on a linin network of large extent, while after the formation of the spindle the chromatin is insignificant in amount. There is here either a great condensation or an actual reduction in the amount of chromatin. Increase in the density of staining favors the former view, although the decrease in volume is too great to be attributed wholly to such a cause.

The nuclear membrane is gelatinized as early as the conditions shown in *figs. 5* and *6*. With the constant shrinkage of the nucleus it decreases in superficial area, but apparently not in volume, inasmuch as it constantly grows in thickness. Eventually the spindle, by the constant shrinkage of the gelatinous membrane, comes to lie in a narrow court surrounded by a broad dense zone of granular substance staining strongly with the orange G. This halo, clearly the residue of the altered nuclear membrane, is a conspicuous object in the field, though the nucleus itself is now very small, averaging 10 μ or less in length. The nucleolus persists unchanged in character from the condition shown in *fig. 10*, *i. e.*, it is similar to the nuclear vacuole shown in *fig. 3*.

A distinct view, slightly after metaphase, is given in *fig. 12*,

showing the chromosomes at the two ends of the spindle. They are probably four in number, although we do not assert this with certainty. After the polar migration of the chromosomes the whole spindle lengthens much, giving a peculiar distorted figure similar to that found in the nuclei of *A. Bliti*, and the telophase is of similar nature to that described for that fungus (Stevens, '99). The spindle fibers fall together in the center and divide, giving rise to the independent daughter nuclei.

This mitosis agrees well in late anaphase with that of many other fungi, conspicuously so with Albugo. In many other respects it is unique. The early dissolution of the membrane and the persistence of its remains as a granular halo around the metaphase and anaphase figures is a new phenomenon, as is also the mode of spindle formation. The spirem also differs from any previously described, and the behavior of the nucleolus is unique; while the disposition of the chromatin in resting and early prophase conditions is exceptional.

The great size of the nucleus led to an inference that the mitosis would present, perhaps more clearly than any other type of fungus, the details of spindle formation. This inference was unfounded, since the shrinkage of the nucleus preparatory to mitosis reduces the spindle to moderate dimensions. Moreover the stages are rare to find. Hundreds of samples were examined showing no trace of mitosis, while a very few leaves of the host plant were found exhibiting good stages. When a block of good material is secured it gives abundant cases, however, to prove the existence of mitosis. Still, as there can be obviously only one primary division in each sorus it is an exceedingly slow task to complete a series. All of the figures represented come from sori bearing only one nucleus, and unquestionably represent primary division. Occasional views of the second and succeeding mitosis were had, but here the subject becomes much complicated and discussion is reserved for a later paper.

The significance of the facts observed, such as the enlargement of the nucleus and its subsequent shrinkage, the peculiarities of mitosis, the chromatin changes, etc., will be better interpreted when the other peculiarities in the cytology of this

fungus are described. The Chytridiales have offered an open field for speculation heretofore, and have baffled definite judgment as to their nature and relationship. Fuller knowledge of their cytological peculiarities may lead to a more satisfactory condition.

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EXPLANATION OF PLATES XVI AND XVII.

All figures are from material killed in chrom-acetic acid and stained with Flemming's triple stain. The figures were sketched with an Abbé camera, using a Leitz $\frac{1}{12}$, ap. 1.30, giving an enlargement of 1790 diameters, with the exceptions of *figs.* 1 and 2, which were drawn from a Bausch and Lomb $\frac{1}{8}$, with a magnification of 750 diameters. Plate not reduced in reproduction.

PLATE XVI.

FIG. 1. One parasitized cell in early stage of development, showing large nucleus and nucleolus surrounded by granular cytoplasm; adjacent cells show nuclei and chloroplasts.

FIG. 2. Parasite nearly at end of growing period; nucleus heavy walled, vacuolate; chromatin distributed along nuclear wall, around the nucleolus, and on connecting strands.

FIG. 3. Single nucleus of a stage similar to but slightly later than *fig.* 2, and more highly magnified; nuclear vacuoles prominent, nucleolar substance giving way to vacuoles; chromatin on nuclear wall also arranged in irregular heaps around the nucleus; nuclear wall still definite and firm.

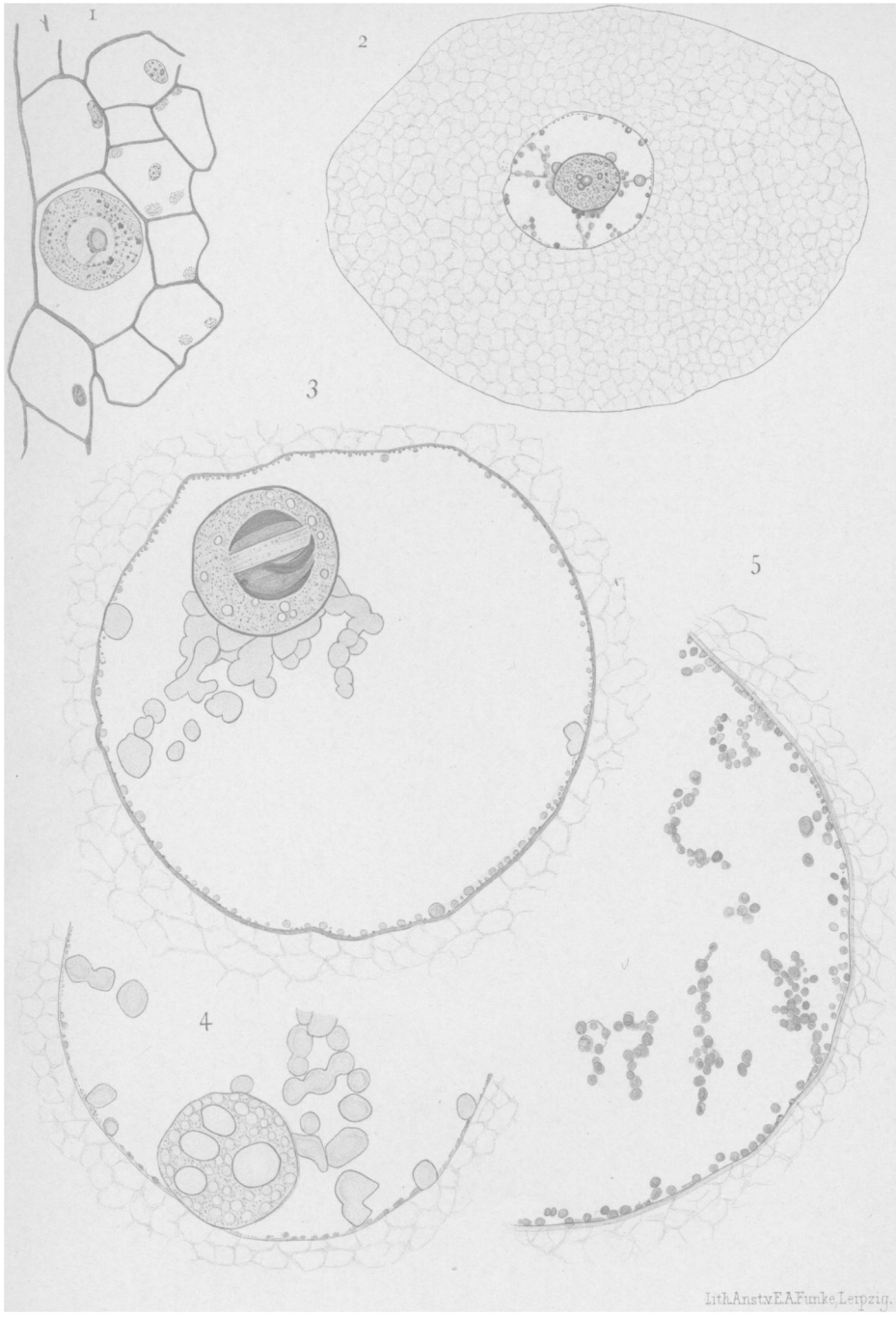
FIG. 4. Portion of nucleus slightly later, showing the continued wasting of the nucleolus and a somewhat more even distribution of chromatin.

FIG. 5. Portions of nucleus still later; nucleolus not present; the large chromatin lumps and globules of *fig.* 4 have given place to more numerous and smaller ones which have also taken on a much more even distribution, thus largely obliterating the large conspicuous vacuole of earlier stages; the nuclear membrane is less distinct on its outer border.

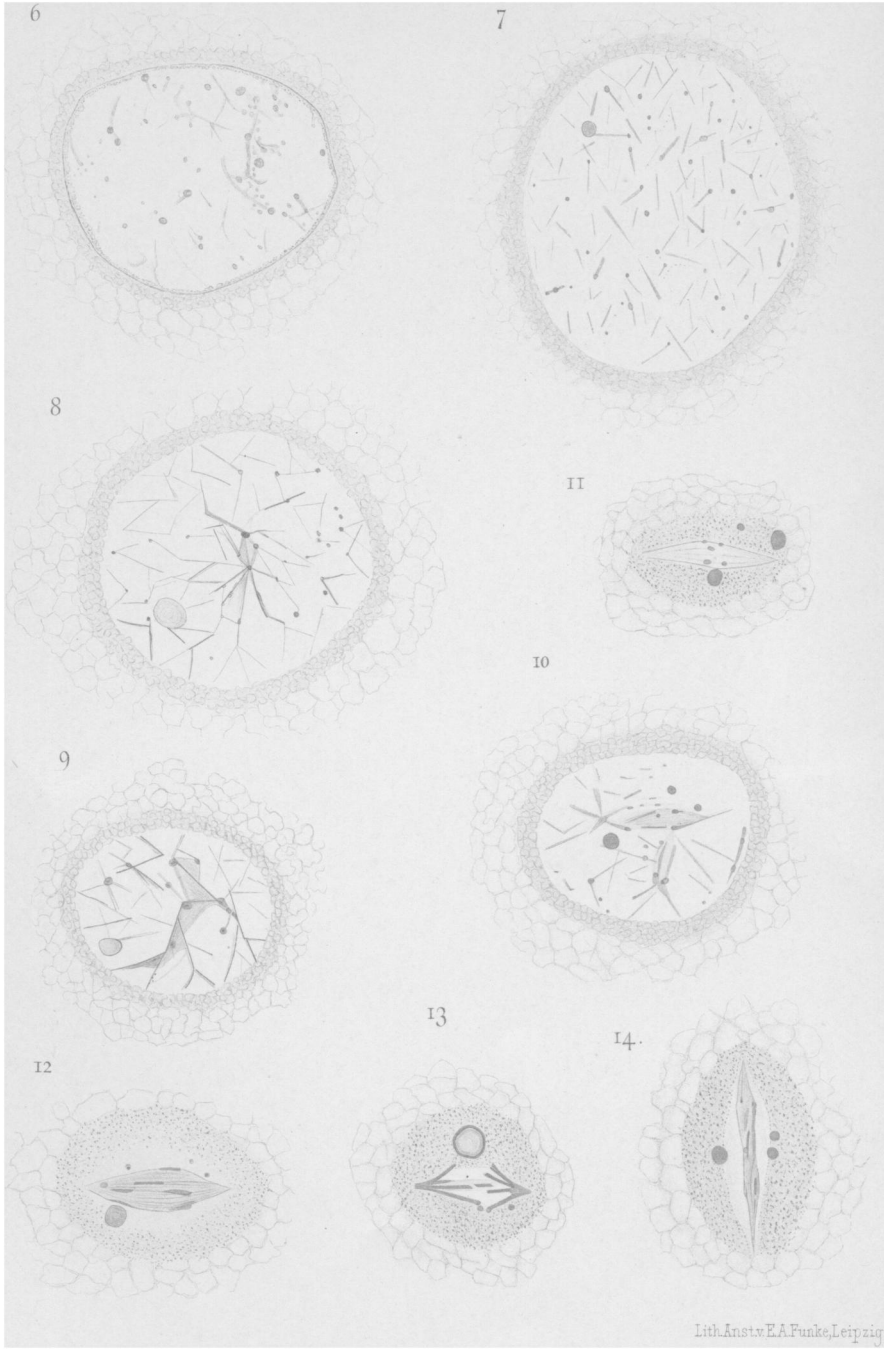
PLATE XVII.

FIG. 6. The chromatin globules of earlier figures are assuming the rod-form; the nuclear membrane has softened from the outside inward, being now largely represented by a thick layer which stains darkly with the orange G.

FIG. 7. Chromatin rods entirely replace the globules of earlier stages; these rods intersect at sharp angles, often appearing as dots from end view; a nucleolus, similar in stain to the nucleolar vacuoles in *fig.* 4, is seen to the



STEVENS on SYNCHYTRIUM



STEVENS on SYNCHYTRIUM

left; a shrinkage in the size of the nucleus is marked in passing from *fig. 3* to *figs. 6–7* and succeeding figures.

FIG. 8–9. The linin commences to aggregate near the center of the nucleus; shrinkage continues and dissolution of the membrane proceeds.

FIG. 10. Linin threads assume spindle form; shrinkage continues and with it increase in the thickness of the layer bounding the nucleus.

FIG. 11. Well defined spindle surrounded by residue of the membrane, now a mass of substance staining densely with the orange G; chromosomes near equator and several nucleoli present.

FIG. 12. Chromosomes passing toward poles.

FIG. 13. Chromosomes at the poles.

FIG. 14. Constriction of the nuclear spindle at the equator preparatory to final separation of the daughter nuclei.

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